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MECHANISM OF MICROCIRCULATORY

FAILURE IN SHOCK

Annual Report
June 1980

by

J. Judson McNamara, M.D.

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REPORT DOCUMENTATION PAGE

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1. REPORT NUMBER	2 GOVT ACCESSION NO	3 RECIPIENT'S CATALOG NUMBER
	A134406	

4. TITLE (and Subtitle)	5 TYPE OF REPORT & PERIOD COVERED
MECHANISM OF MICRO-CIRCULATORY FAILURE IN SHOCK	Annual (9-1-79 to 8-31-80)
	6. PERFORMING ORG. REPORT NUMBER

7. AUTHOR(s)	8 CONTRACT OR GRANT NUMBER(s)
J. Judson McNamara, M.D.	DADA17-73-C-3040

9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
Cardiovascular Research Laboratory Queen's Medical Center Honolulu, Hawaii 96813	62772A.3S162772A874.AB.130

11. CONTROLLING OFFICE NAME AND ADDRESS	12. REPORT DATE
U.S. Army Medical Research and Development Command, Fort Detrick Frederick, Maryland 21701	June 1980
	13. NUMBER OF PAGES
	43

14. MONITORING AGENCY NAME & ADDRESS(If different from Controlling Office)	15. SECURITY CLASS. (of this report)
	Unclassified
	15a. DECLASSIFICATION DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release, distribution unlimited.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

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SUMMARY

A prolonged reduction of visceral organ blood flow has been clearly demonstrated in our laboratory in primates after resuscitation from hemorrhagic shock. This is consistent with observations on visceral function and blood volume changes in humans after successful resuscitation from shock. The mechanism is unclear but appears to be related to persistent abnormalities in the body's vasoregulatory mechanisms both at the systemic (catecholamines and renin/angiotensin II) and local (thromboxane A/prostacyclin) levels. The background for this hypothesis is discussed in detail.

The present proposal using both a primate shock model and a pig hind limb perfusion system begins to evaluate these possible mechanisms and investigates empirical treatment mechanisms as follows:

1) Studies are undertaken on the effects of controlled vasodilator therapy on our primate shock model.

2) Determinations of hormone levels relative to production of catecholamines, renin and angiotensin II and Thromboxane A_2 and prostacyclin are undertaken over an 18-hour period in our primate shock model.

3) Hemodynamic and metabolic effects of hind limb perfusion with blood from an animal 8 hours after resuscitation from hypovolemic shock.

Finally, hypertonic glucose is investigated as a stop-gap measure for preserving life in primates in hemorrhagic shock. The rationale for this concept is well supported from recent research on the benefits of supplying high dose glucose as an acute energy substrate in shock states in man.

PART I: BLOOD FILTERING

During the past year we have expanded our study on blood filtering efficiency in a clinical setting. The Intercept filter (Johnson & Johnson) went off the market and was dropped from the study. A summary of the results of all of the data collected from this study are contained in Appendix A. A further study was carried out in a group of multiple trauma patients receiving massive transfusion. This data is not yet complete but will be completed by the end of the current contract period and further studies of blood filtration will not constitute a part of the contract renewal proposal.

Several conclusions appear justified:

1) In terms of clinically significant pulmonary insufficiency the problem was rare after surgery or trauma in the present series. No filter made a significant difference in the small incidence of pulmonary insufficiency in the present series.

2) Certain filters were a bit more difficult to set up and had a higher incidence of problems with initiating flow early in the study. However, with experience all filters appeared about the same in terms of rapidity with which effective flow could be established.

3) Microfilters significantly slowed the rate of blood administration. The magnitude of the slowing was not great, however. Pall and Travenol filters appeared to have a slightly higher capacity than other filters studied but these differences are not significant.

4) It is evident from the present data that, although filters didn't seem to prevent any identifiable clinical problem with massive transfusion, they do significantly interfere with the rate of blood infusion and consequently inhibit the effectiveness of resuscitation of the injured patient.

The issue remains as to whether or not to use ultrafilters.   The facts are:

1) Stored blood contains microaggregates of biological debris.

2) Although the debris has not been shown to produce any harm, this may in part be due to the insensitivity of the biological models utilized. Certainly, it appears to do no good and for a number of theoretical reasons could be harmful.

3) Ultrafilters will remove the debris and do not appear to damage the blood but do slightly interfere with the rate of resuscitation by slowing the rate of blood infusion.

Consequently, based on the present data, ultrafilters cannot be recommended for use in blood transfusions.

PART II: TISSUE PERFUSION AFTER RESUSCITATION FROM HEMORRHAGIC SHOCK

We have studied three groups of primates with a standard hemorrhagic
shock model. The first was completed under the previous year's grant and
the latter two during the past year. A summary of the data in these three
groups is contained in Appendix B. In short, we have shown that 18 hours
after resuscitation from hemorrhagic shock blood volume remains significantly
below baseline levels (10-15%) in spite of otherwise baseline hemodynamic data.
Furthermore, regional visceral organ blood flow to the lungs (systemic), liver,
intestine, stomach, kidney and spleen are significantly below baseline values
at 18 hours, whereas heart and brain blood flow are normal. Hemodynamic para-
meters are normal.

We needed a reproducible, stable control model in which we could use a
single hemodynamic parameter as a therapeutic index of the dosage of nipride
administration as an intervention in the shocked animal. We did a group of
animals in which, after successful restoration of MAP and LAP, fluid replace-
ment was only given at maintenance levels for the next 16 hours. During this
period there was a gradual decrease in all hemodynamic parameters and blood
volume was even more reduced at 18 hours. (Appendix BII).

Since we were still unable to establish a consistent drug administration
and volume replacement criteria for this model as we had anticipated using
baseline LAP as the criteria for adequate volume replacement and then an arbi-
trarily chosen 25-30% reduction in systolic pressure as the therapeutic criteria
for nipride administration. It required huge volumes of fluid to maintain LAP
of which was promptly excreted by the kidneys with an end result that at 18
hours, although hemodynamic parameters remained normal, visceral organ flow
and blood volume were still reduced at 18 hours. (Appendix BIII).

Since LAP could not be used as a resuscitative endpoint, we undertook
further studies during the past year to establish a reproducible control model.
Two additional animals in this group were treated with nitroprusside for about
2 hours after the shock period. The criteria for drug dosage was difficult
to manage but the preliminary data suggested that the persistent abnormality
in visceral blood flow might be effected by controlled, early vasodilatation.

Consequently, we repeated the above experiment using MAP ± 10% as the
criteria for adequate resuscitation. This group of animals produced consistent,
reproducible hemodynamic data and confirmed the persistent reduction in visceral
blood flow and blood volume in spite of apparently perfectly adequate resus-
citation to baseline hemodynamic values. (Appendix BIV).

We now need to undertake two tasks in this project:

1) Complete a group of nipride treated animals to assess our original
hypothesis that prolonged selective vasomotor disequilibrium is responsible
for the maldistributed visceral blood flow.

2) To evaluate the mechanism for the prolonged maldistribution in blood
flow which has been consistently documented in all three of our study groups.

PART III: REVIEW OF ISOLATED LIMB PERFUSION STUDY TO DATE

In this study we found that perfusion of an isolated pig hind limb with autologous hemorrhagic shock blood resulted in a significant increase in peripheral vascular resistance compared to perfusion with autologous normal (non-shock) blood. This increased resistance could be eliminated with phentolamine. However, oxygen consumption remained depressed during perfusion with shock blood in spite of normal flows ($p < 0.05$, one tailed t test for paired data). (Appendix C). Because of the small sample size (8 pigs) and the marginal statistical significance we continued the study to verify the findings and also measured blood transit time through the limb to evaluate the possible role of arteriovenous shunting during perfusion with shock blood.

Thirty-two limb perfusions have now been evaluated, including one dog and one calf. In a total of 15 controls, including the dog and calf (Table I), peripheral vascular resistance increased from 1.0 resistance units during perfusion with normal blood to 2.1 resistance units during perfusion with shock blood ($p < 0.001$).

In a total of 17 phentolamine treated pig limbs peripheral vascular resistance was the same for normal and shock blood (Table II). Oxygen consumption dropped from 1.52 ml/min during perfusion with normal blood to 1.24 ml/min during perfusion with shock blood, but this difference in this larger group is no longer statistically significant at the 0.05 level for P (Table III).

In 9 of the 17 phentolamine treated pig limbs blood transit time through the limb was evaluated by the dye dilution method using cardiogreen and a densitometer (Table IV).

Transit time was evaluated by three methods: (1) time of first appearance of the dye in the venous effluent, (2) time of the maximum dye appearance in the venous effluent, and (3) the mean transit time of the dye evaluated by using the best log fit for the downslope of the curve.

The time of first appearance was 23 seconds for normal blood compared to 24 seconds for shock blood. The time of maximum dye appearance was 49.3 seconds for normal blood and 47.5 seconds for shock blood, and mean transit time was 82.4 seconds and 79.4 seconds for normal and shock blood respectively. The corresponding values for oxygen consumption were 1.5 ml/min for normal blood and 1.4 ml/min for shock blood. None of these differences was statistically significant.

The above findings show that phentolamine can eliminate the increased peripheral vascular resistance found in control limbs perfused with shock blood. They also suggest that there is no significant arteriovenous shunting

occurring during perfusion with shock blood treated with phentolamine. In addition, the above results suggest that, in contrast to our previous findings, phentolamine can apparently also return oxygen consumption in the isolated limb perfused with shock blood to within normal or nearly normal values.

Although elevated catecholamines were documented in the shock blood, phentolamine is a direct vasodilator as well as alpha adregergic blocking agent and thus could be producing effects other than merely blocking catecholamines.

This data also demonstrated that the hind limb preparation is a sensitive model for assessing the immediate neurohumoral (i.e., catecholamine) responses to shock.

Table I.   Summary of PVR During Perfusion With
Normal and Shock Blood in 15 Controls

		Normal Blood	Shock Blood
ILP-20	(control)	1.1	1.9
21	(control)	1.0	1.9
22	(control)	.8	1.6
23	(dog)	.5	.6
24	(control)	1.1	2.4
25	(control)	.5	1.2
26	(control)	.6	3.4
27	(aspirin)	1.4	3.1
29	(aspirin)	1.2	1.9
30	(cell separator)	.9	2.4
32	(preheparinized)	1.6	2.9
33	(cell separator)	1.0	2.0
34	(cell separator)	.9	2.0·
35*	(phentolamine)	.9	1.6
38	(calf)	1.0	2.5
Mean ± S.D.		1.0 ± .3	2.1 ± .7

t=7.05
(p<0.001)
2-tailed

* Included in both controls and phentolamine because phentolamine
was added to shock blood after seeing the usual ↑ PVR. The PVR
shown here is before adding phentolamine.

Table II.   ILP

Summary of PVR and $\dot{V}O_2$ During Perfusion With
Normal and Shock Blood in Phentolamine Treated Limbs

	(n=17) PVR		$\dot{V}O_2$ (ml/min)	
	Normal Blood	Shock Blood	Normal Blood	Shock Blood
ILP-35	.9	.8	1.1	.8
36	1.3	1.3	1.5	.9
37	1.5	1.6	2.4	2.2
40	.6	.6	1.0	.9
41	.6	.7	2.4	.9
45	.8	.8	. 1.6	1.6
48	1.3	1.3	.7	.5
53	1.2	1.2	1.6	1.6
56	1.3	1.4	1.0	1.0
57	1.0	1.1	2.1	1.7
59	.5	.7	2.1	2.6
60	.6	.6	2.1	.9
67	.9	.9	1.7	.9
68	1.1	1.3	1.5	1.8
70	1.2	1.5		1.6
73	.7	.7	.9	1.1
74	.8	.8	1.0	1.2
Mean ± S.D.	1.0 ± .3	1.0 ± .3	1.5 ± .5	1.3 ± .6

(t = 1.6)
n.s.

Table III.

In 9 of the 15 controls $\dot{V}O_2$ was calculated for normal and shock blood and is shown below with flow and PVR.

		$\dot{V}O_2$ (ml/min)		Q (ml/min)		PVR	
		N	S	N	S	N	S
ILP-20	(control)	.6	.1	50	16	1.1	1.9
21	(control)	.8	.4	60	20	1.0	1.9
22	(control)	1.1	.8	65	37		1.6
24	(control)	.9	.6	47	21	1.	2.4
25	(control)	1.5	.9	89	41	.	1.2
26	(control)	1.7	.7	70	16	.6	3.4
27	(aspirin)	1.1	.7	32	13	1.4	3.1
29	(aspirin)	1.1	.9	38	19	1.2	1.9
38	(calf)	4.6	1.7	50	20	1.0	2.5
Mean ± S.D.		1.5±1.2	.8 ± .4	56 ± 17	23 ± 10	1.0 ± .3	2.2 ± .7

$t = 2.6$ $t = 8.1$ $t = 5.4$
$(p<0.05)$ $(p<0.001)$ $(p<0.001)$
2-tailed   2-tailed   2-tailed

Table IV.   ILP - Transit Time

(Calculated in 9 of 17 Phentolamine limbs)
(n = 9)

	First Appearance of Dye		Time of Maximum Dye Appearance		Mean Transit Time	
	Normal Blood	Shock Blood	Normal Blood	Shock Blood	Normal Blood	Shock Blood
ILP-56	34	28	66	57	100	85
57	22	20	42	41	129	80
59	16	15	28	27	40	51
60	16	16	29	30	54	47
67	22	23	44	41	66	60
68	24	28	84	72	106	119
70	34	38	74	78	133	144
73	20	20	38	40	55	58
74	19	22	37	41	58	69
Mean ± S.D.	23 ± 7	24 ± 7	49 ± 20	49 ± 18	83 ± 35	79 ± 33
	T = 1.8		T = 2.9		T = 0.44	

Corresponding $\dot{V}O_2$ shown in Table II.

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BIBLIOGRAPHY

Article

Pressler VM, Suehiro A, Lum J, Mori K, McNamara JJ: Mechanisms of shock
blood induced tissue anoxia.   Am J Surg 140:47, July 1980.

Abstract (unpublished data)

McNamara JJ, Suehiro G, Suehiro A, Jewett B: Abnormalities in visceral
blood flow after resuscitation from hemorrhagic shock.

APPENDIX A

Blood Filter Data (Preliminary Data)

The collected data and date on the clinical assessment of blood filters
is included here. Table I compares the variables involved in the mechanics
of the filter system which significantly (p<0.05) independently influenced
the flow rate as analyzed by multivariation analysis. The impact of the
variable is listed in order of decreasing significance. Thus, the factors
which influence the rate of blood infusion most significantly are smaller
needle size, older blood, the use of gravity flow rather than pressure
infusion, the absence of a blood warmer, the previous use of the filter for
infusion of blood and the use of an ultrafilter.

Fig. 1 shows that flow rates of blood through filters do not distinguish
significantly between filters. Fig. 2 depicts data relating to the time
necessary to set up blood filters. Although the data is preliminary the
Pall, Standard and Swank filters all are set up in about the same amount of
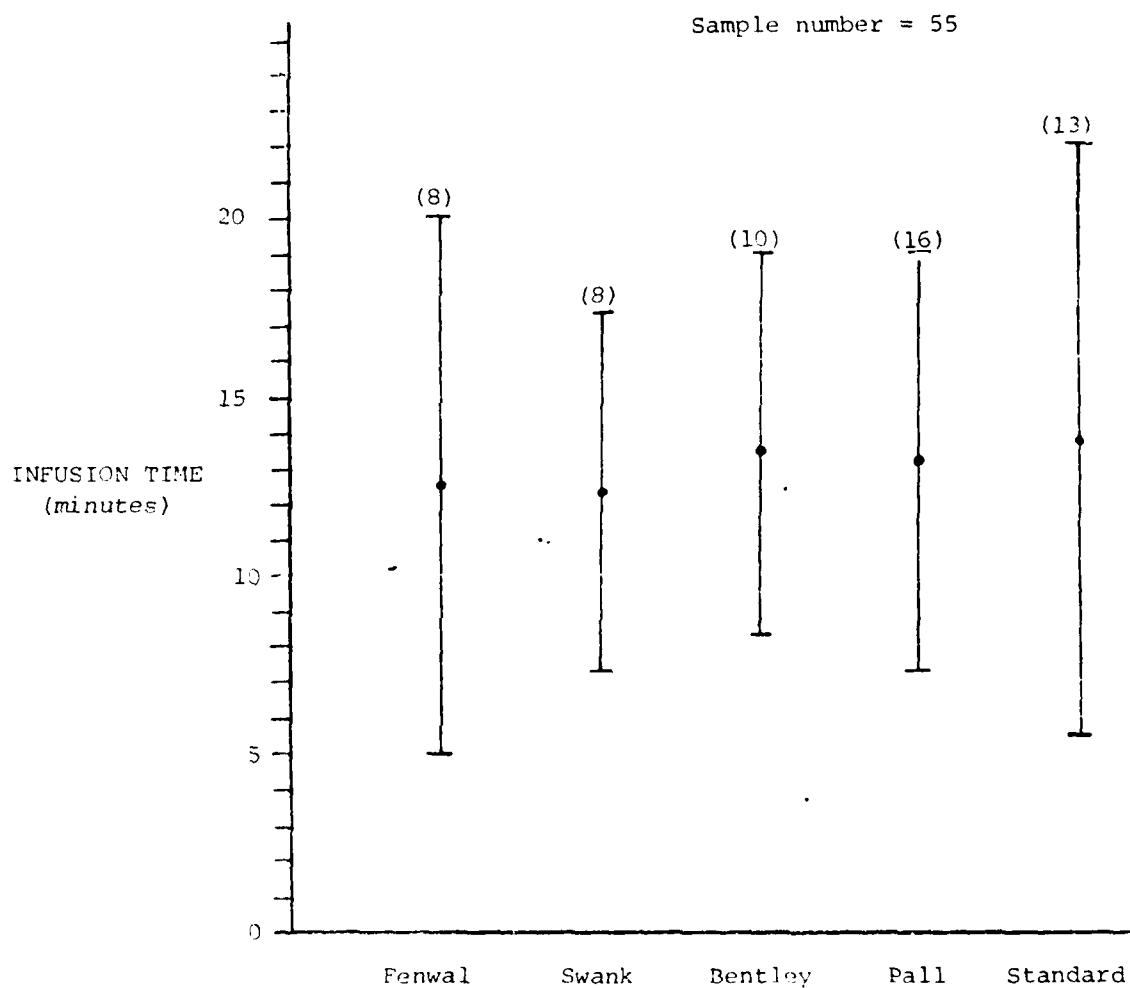time.

Blood Filter Study

06/13/80

DEPENDENT VARIABLE.   INFUSE   M U L T I P L E   R E G R E S S I O N
                              INFUSION TIME MINUTES

VARIABLE LIST 1
REGRESSION LIST 1

SUMMARY TABLE

VARIABLE		MULTIPLE R	R SQUARE	RSQ CHANGE	SIMPLE R	B	BETA
Needle	Needle gauge	0.58646	0.34394	0.34394	0.58646	7.257755	0.52378
Age	Age of blood (days)	0.64679	0.41834	0.07440	0.30239	1.692293	0.25215
Flow type	Gravity or pressure	0.68842	0.47392	0.05558	-0.36071	-18.07929	-0.23811
Warmer	Blood warmer	0.68994	0.47601	0.00209	-0.20785	04.900790	-0.04487
Prev unit	Previous nits through filter	0.69032	0.47654	0.00053	-0.11313	1.086687	0.02459
Nfilt	Micro or STD filter	0.69051	0.47680	0.00026	0.05019	2.545784	0.01623
(Constant)							

INFUSION RATES OF PRESSURE INFUSED WHOLE BLOOD

Sample number = 55

INFUSION TIME
(minutes)

The above is the mean and standard deviation or the first unit of whole blood infused through each filter.

SET-UP TIME

NUMBER OF
SET-UPS

15
14
13
12
11
10
9
8
7
6
5
4
3
2
1

J&J — 10, 3
Fenwal — 5, 8
Bentley — 5, 8
Pall — 2, 7
Standard — 1, 6
Swank — 2, 4
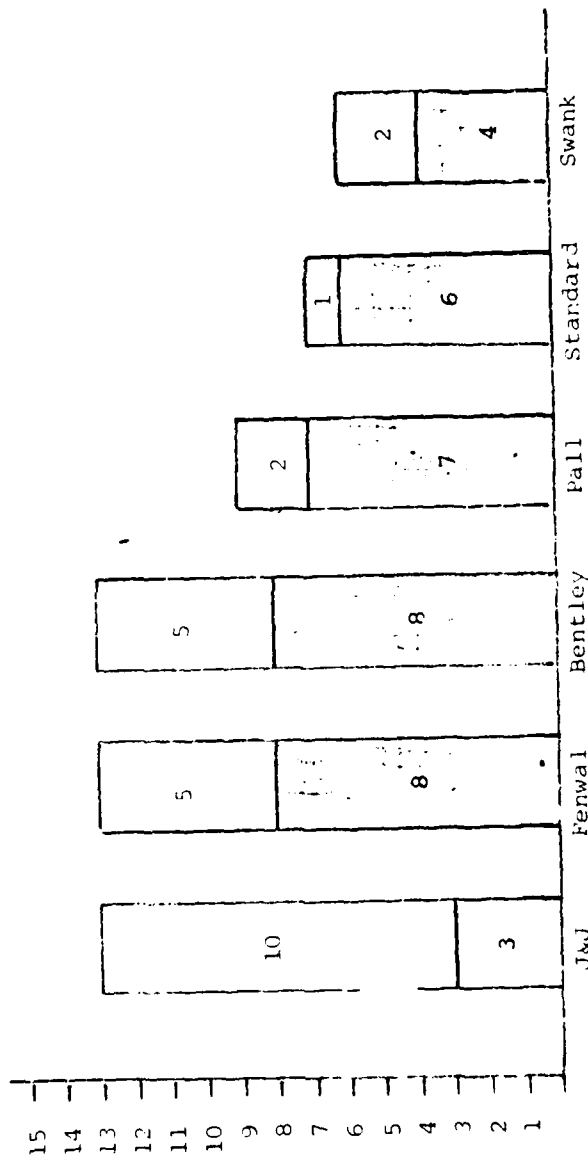
☐ = No. set-up in >1 minute

☐ = No. set-up in <1 minute

Set-up time = This is documented time that each anesthesiolgoist spent taking each filter from its packaging to the time blood began infusing into the patient.

APPENDIX B

Section I   A summary of the general methodology for conducting
                 our shock experiments.

Section II   A summary of the initial shock group animals
                 including 6 control animals and 2 treated.

Section III   A summary of 8 animals in which LAP was used as
                 the index of resuscitation.

Section IV   A summary of 6 animals in which MAP was used as
                 an index of resuscitation.

Appendix BI - A working summary outlining the general steps involved in
carrying through a shock experiment with our current primate
model

APPENDIX BI

Protocol

The basic protocol for this set of experiments is outlined below. The only variation between groups exists at step 13 involving resuscitation of the animal and this variation will be discussed for each group.

1. Anesthesia*: Administer 5 ml of sernylan (3.3 mg/ml) or ketamine to baboon IM. Use pentothal (IV) when required.
2. Shave animal and wash.
3. Obtain weight (kg).
4. Insert urine catheter.
5. Intubate
6. Attach EKG electrodes
7. Perform cutdowns (sterile), cannulating both femoral arteries and veins (CVP).
8. Initiate left thoracotomy.
   a. Place stiff wall pressure line in_ᵣ left atrium.
      1) Adjust height of 2A transducer for zeroing.
   b. Attach pulmonary artery flow probe.
9. Close all incisions.
10. Hook IV line to 500 ml bottle of 5% Dextrose in 0.45% saline plus 20 mEq KCl.
11. Baseline measurements.
    a. Serum lactate: not less than 1 ml into grey top tubes (potassium oxalate, sodium fluoride).
    b. Arterial and venous blood gases.
    c. Cardiac output, mean arterial pressure, left atrial pressure, central venous pressure.
    d. Hemoglobin.
    e. Tissue blood flow: 800,000 or more microspheres (Ce, Cr, Sr, Sc).
    f. Intravenous fluids.
    g. Urine output.
    h. Blood volume: Evans blue technique.
12. Induce shock.
    a. Rapidly drop pressure to 60 mmHg for 1 hour.
    b. Exsanguinate until 40 mmHg. Maintain at 40 mmHg for 1 hour.
    c. Obtain parameters 11a-11g.
    d. Retain blood in heparinized syringes.
13. Resuscitation.
    a. Return blood, using last drawn blood first.
    b. Infuse blood unᵣᵢₗ FAP eugals baseline value (+15%).
    c. Maintain LAP with remaining shed blood and Ringer's injection.
14. Two hours post-resuscitation.
    a. Obtain measurement; 11a-11g.
15. Eighteen hours post-resuscitation.
    a. Obtain measurements 11a-11h.
16. Sacrifice animal.
    a. Obtain organ samples for scintillation counting.
       1) Brain, heart, intestine, kidney, liver, lung and spleen

* During shock: use no drugs of any kind.
  During post-resuscitation: use only valium (IV) or ketamine (SC).

Appendix BII

Eight animals were subjected to shock, resuscitated with shed blood
and then treated with a fixed fluid volume replacement with crystalloid
over the next 18 hours (50 ml/kg Ringer's lactate). Two animals, in
addition, received nitroprusside (1.6 ugm/kg/min) as required to keep
mean arterial pressure at a level of 70% of baseline.

Fig. 1 shows the MAP over the entire study period. Maintenance fluids
resulted in a gradual decline in MAP or the 18 hours post resuscitation.
This did not occur in the two treated animals. Fig. 2 shows regional blood
flow values determined at 4 times during the experiment. Significant
decreases were noted in all organs except the brain during shock and in
the kidney, liver and spleen at 18 hours. Significant increases in blood
flow occurred at 2 hours in the brain, heart, intestine and lung. Cardiac
output (Fig. 3) was higher in the control group as compared to the two
nipride animals at 1 hour post resuscitation.

Fluid balance and blood volume changes showed a higher urine output
in the nipride treated animals. Also, there was a greater discrepancy
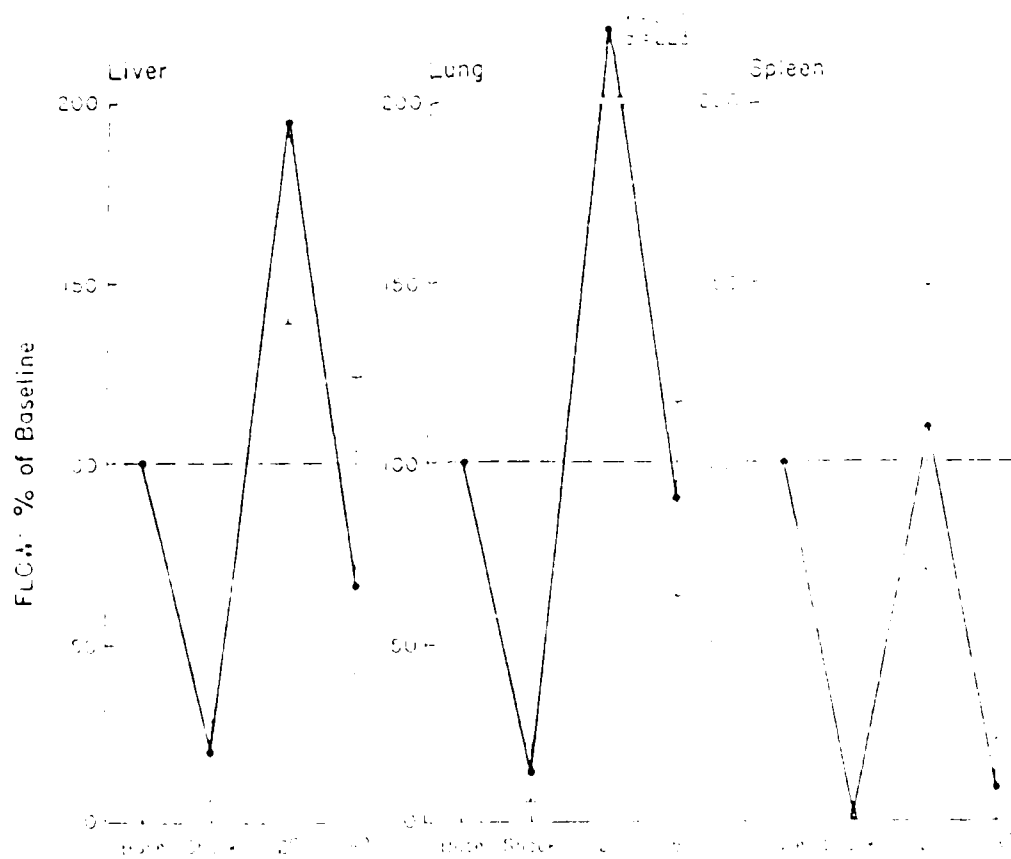(decrease) in blood volume after resuscitation in the control animals.

Control n = 6
Treated n = 2

HOURS POST RESUSCITATION
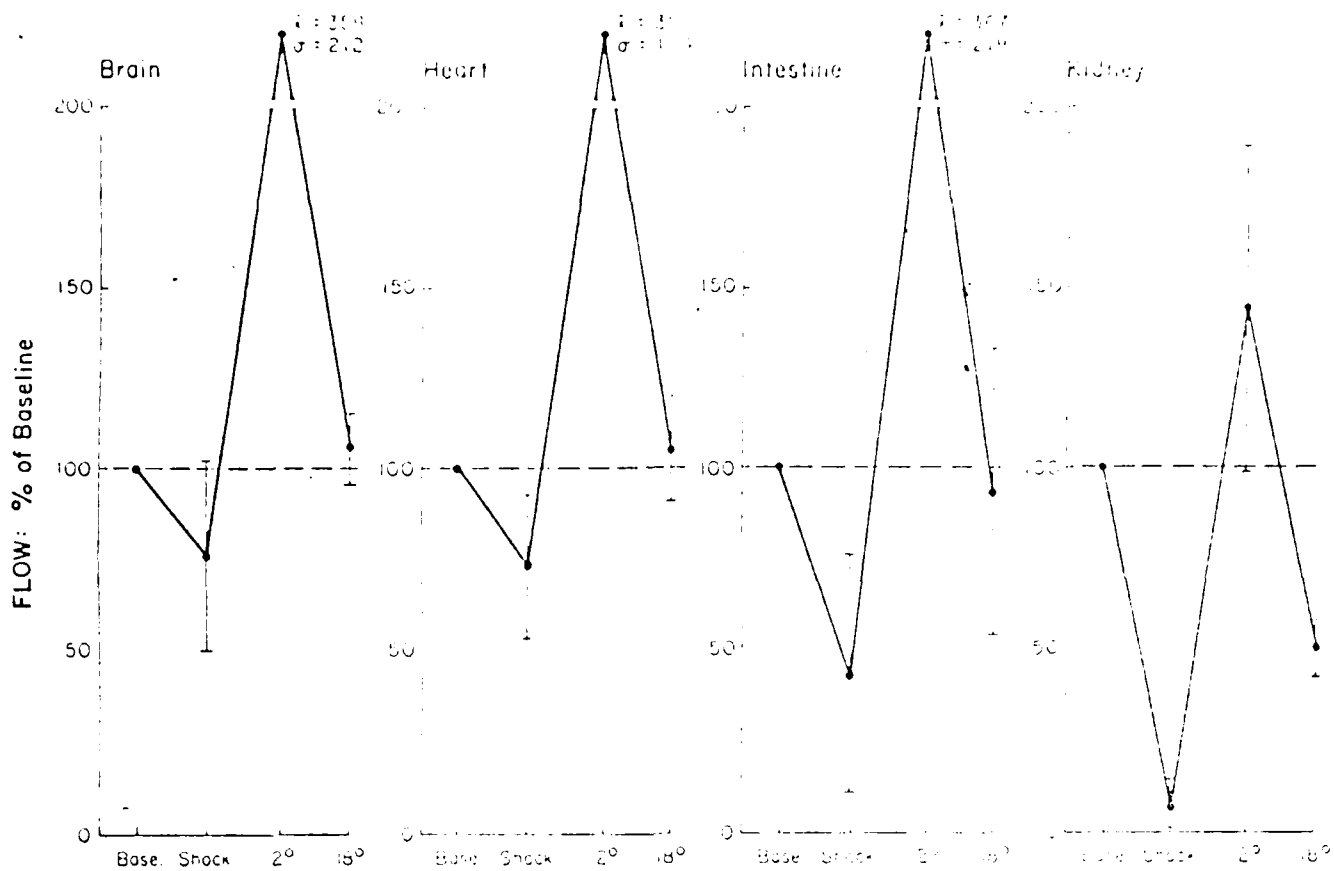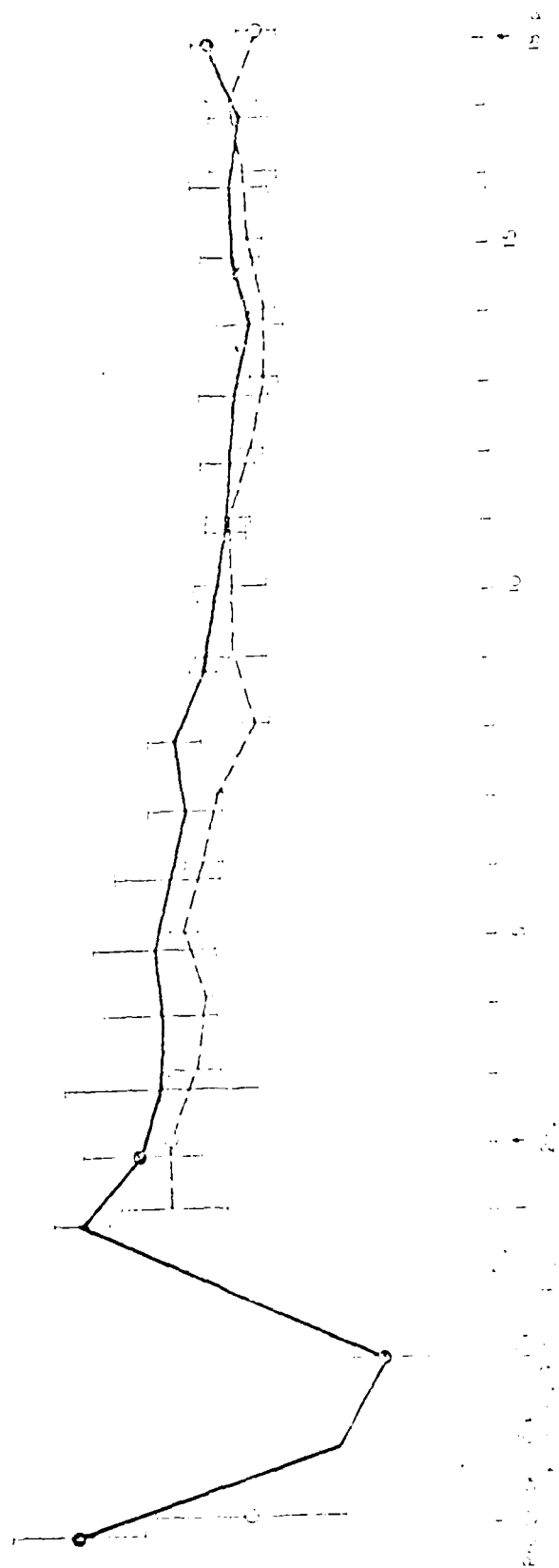
Figure 1

FAP (mm Hg)

Figure 2

CARDIAC OUTPUT (liters/kg)
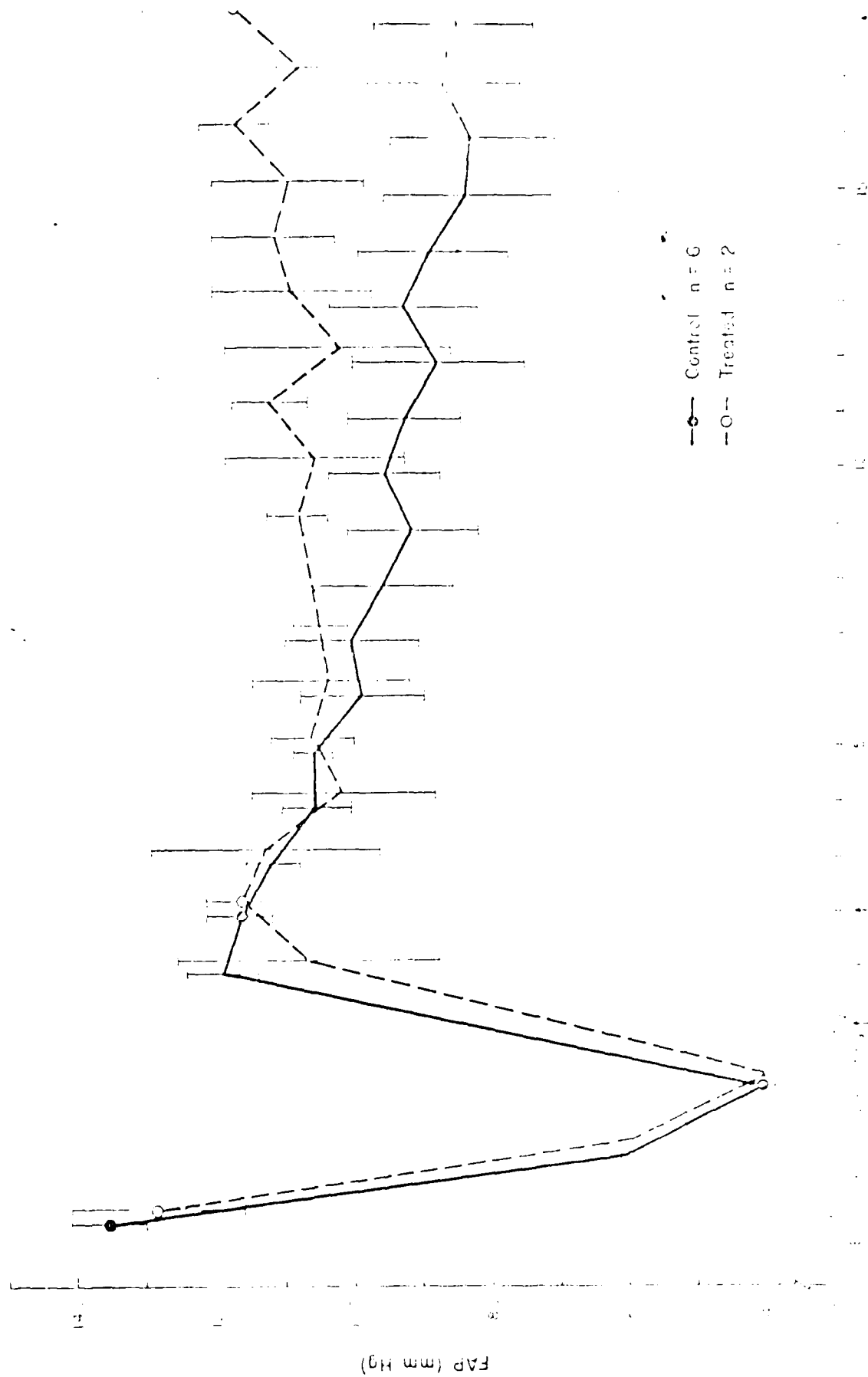
Control n = 5
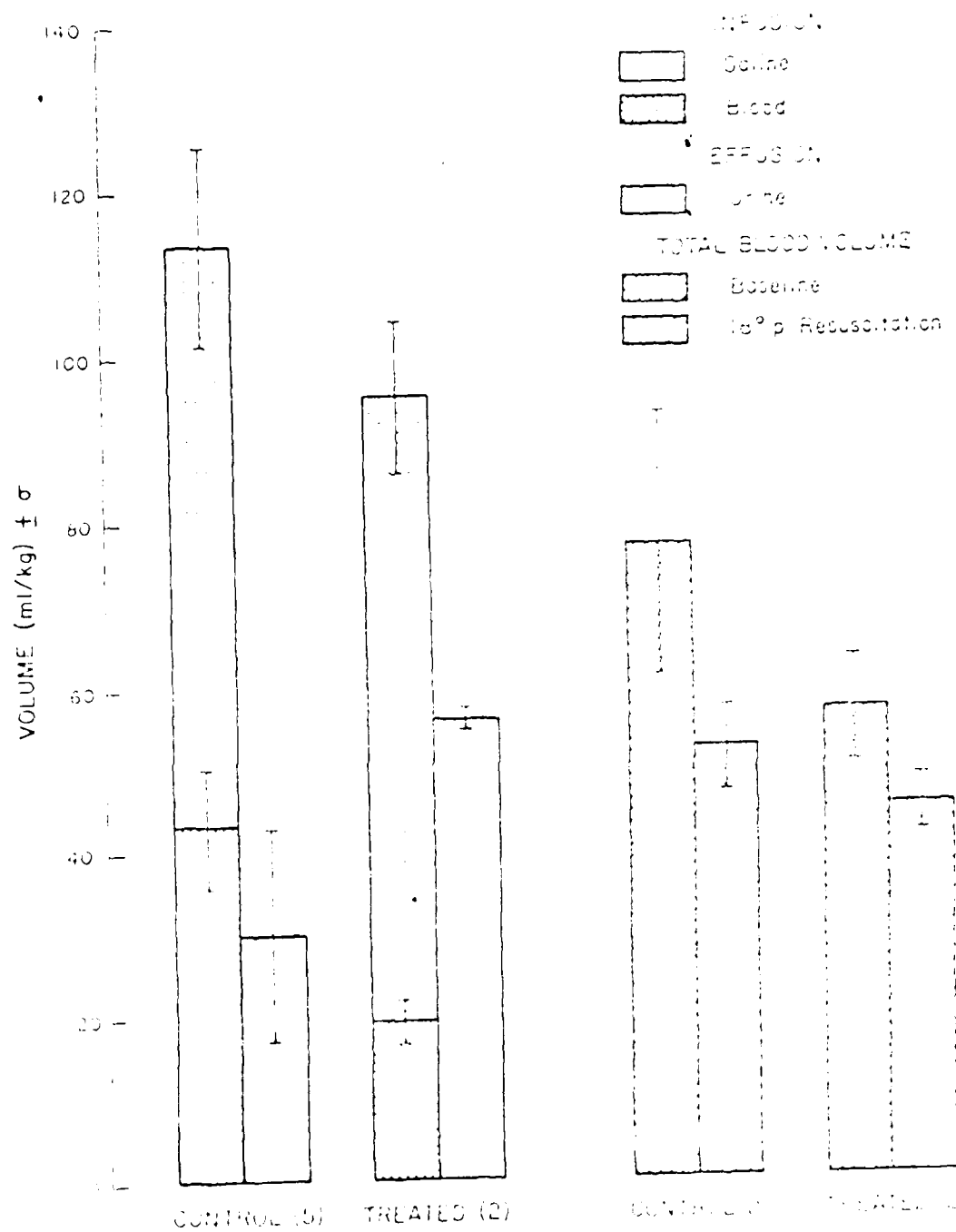
Treated n = 2

HOURS POST RESUSCIATION

Figure 3

Figure 4

APPENDIX BIII: Resuscitation From Shock

     Group II: Eight baboons were bled into shock and maintained at 60 mmHg
and 40 mmHg for 2 hours respectively followed by resuscitation with shed blood
and Ringer's lactate. Restoration of baseline left atrial pressure (LAP) was
considered complete resuscitation and was maintained at baseline values with
infusion of Ringer's for 18 hours. Complete hemodynamic parameters were
recorded pre-shock, during shock and hourly post resuscitation for 18 hours.
Organ blood flow was measured from radioactive microsphere injection at base-
line, during shock and 2 and 18 hours post resuscitation. Blood volume was
determined (Evans blue) at baseline and 18 hours. Characteristic hemodynamic
changes were noted with shock which returned to normal for the 18 hours post
resuscitation (Figs. 1 and 2). Using LAP as the parameter for resuscitation,
increasing fluid requirement to maintain LAP, began at 3 to 4 hours and was
paralleled by a corresponding increase in urine output (Fig. 2). At 18 hours
intake was 450 cc/hour and output nearly 350 cc/hour (both = 4 times baseline).
Organ blood flow altered as expected, during shock. At 18 hours persistent,
significant ($p < 0.05$) reduction in organ blood flow was noted in the portal
circulation (gut and spleen) (Fig. 3). Blood volume was significantly below
baseline (<90%) at 18 hours (Fig. 4). The data suggests persistence of the
sympathetic response in spite of hemodynamic resuscitation. Furthermore, the
excessive fluid intake and output necessary to maintain baseline LAD suggest
resetting of normal mechanisms for regulation of blood volume. Maintenance of
normal LAP may then not be a reliable parameter of resuscitation from hemorrhagic
shock. These observations describe a prolonged abnormality in blood volume and
flow distribution which may contribute to the syndrome of multiple organ failure
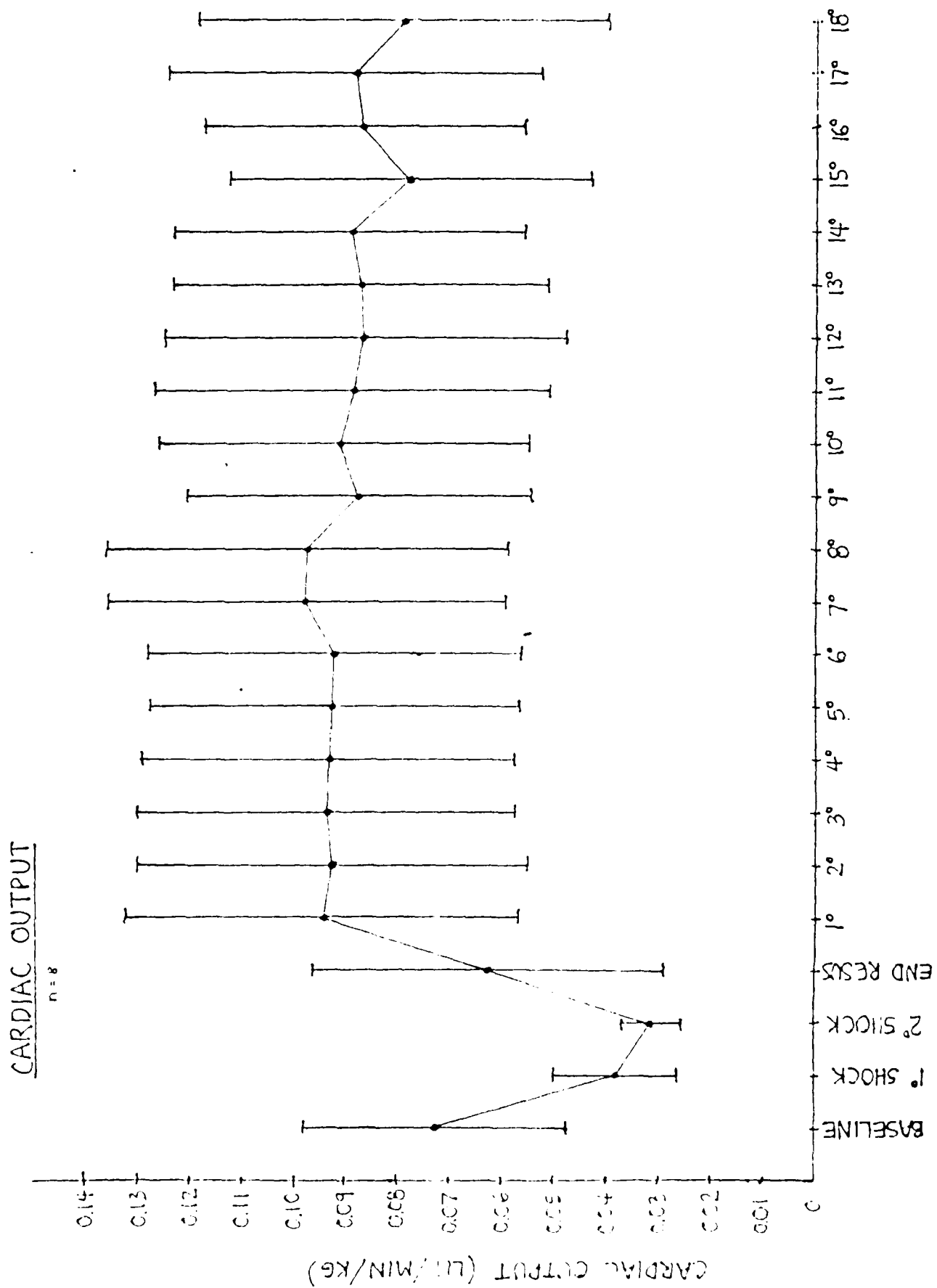following resuscitation from shock.
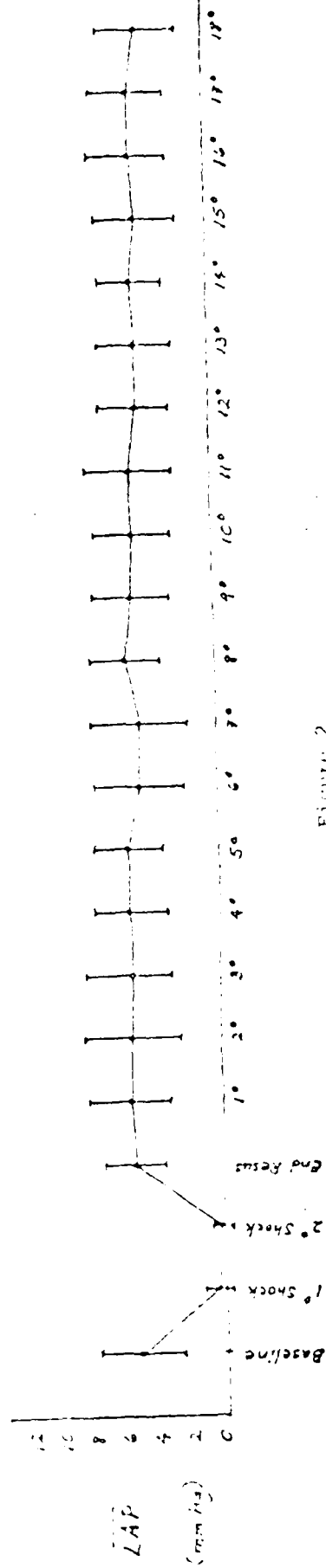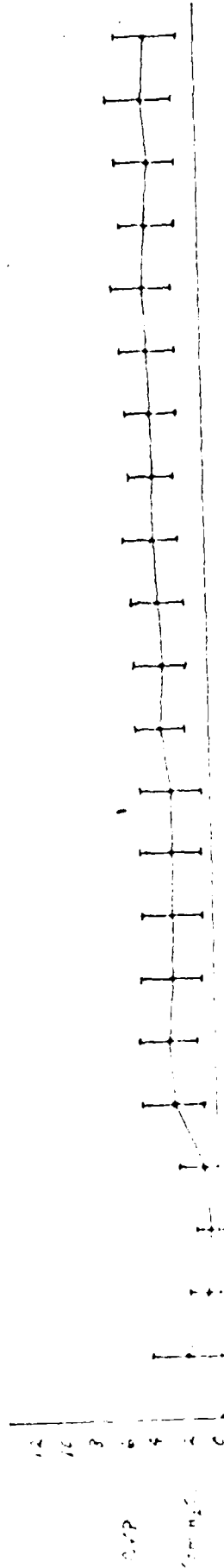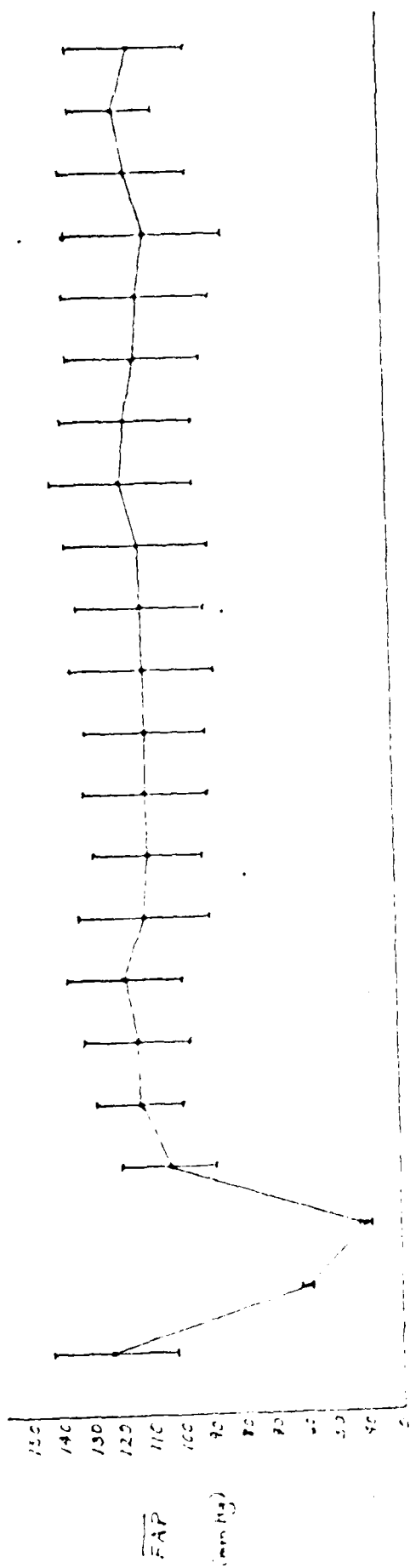
Figure 1

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PRESSURES
n = 8

FAP
(mm Hg)

135
140
130
120
110
100
95
90
70
60
50
40
30

LAP
(mm Hg)

Baseline   1° Shock   2° Shock   End Resus   1°   2°   3°   4°   5°   6°   7°   8°   9°   10°   11°   12°   13°   14°   15°   16°   17°   18°

Figure 2

MICMA ... Tissue ...

n = 8

Brain
Flow: % of Baseline

Base   Shock   2°   18°

Heart
Base   Shock   2°   18°

Intestine
Base   Shock   2°   18°

Kidney
Base   Shock   2°   18°

Liver
Flow: % of Baseline

Base   Shock   2°   18°

Lung
Base   Shock   2°

Muscle
Base   Shock   2°   18°

Spleen
Base   Shock   2°   18°
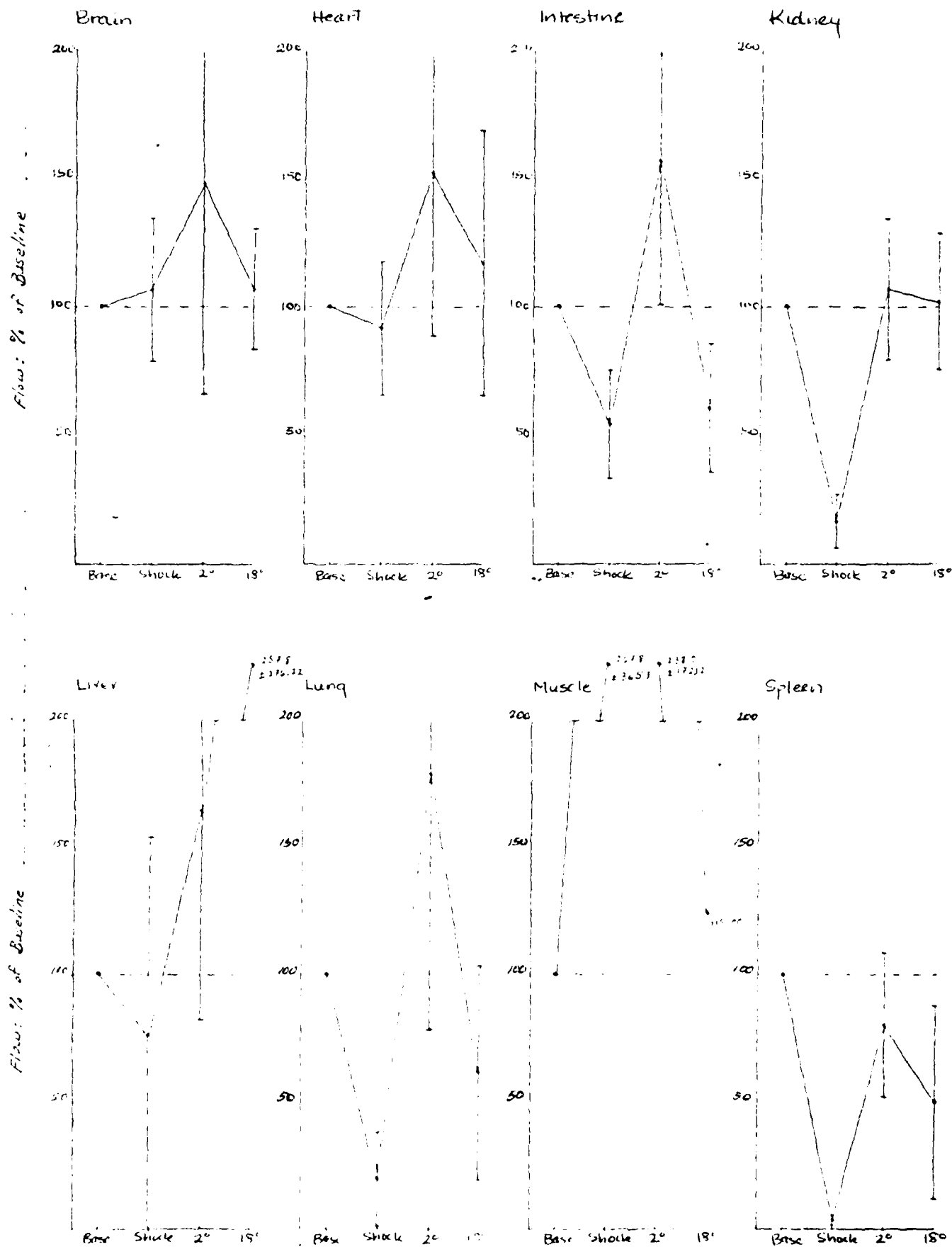
Figure 3

Fluid Dynamics
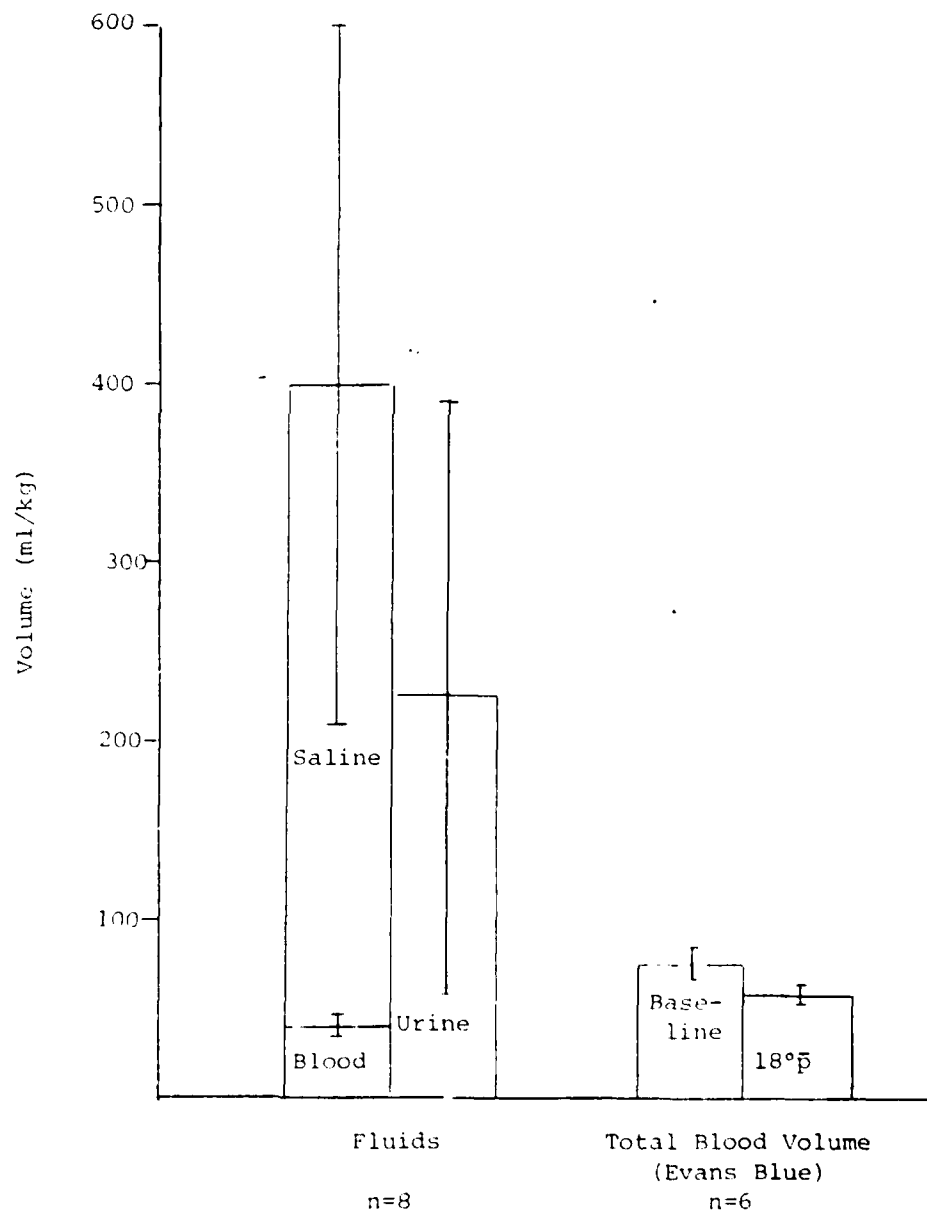
Fig. 4

Appendix BIV - The current control group consists of 6 animals, one sham and 5 controls in which resuscitation has been to a mean arterial pressure of ±10% base MAP and maintained at that level. Hemodynamic data appears more stable (Figs. 1-5) and excessively high fluid intake and output are eliminated (Figs. 1-5). This experimental group is not completed so the hemodynamic data have not been evaluated statistically and the blood flow and blood volume data are incomplete. This appears to be a much more satisfactory model, however, for assessing the effectiveness of different resuscitative therapies. A sham animal was carried out to document the effect of the non-shock aspects of the protocol (Fig. 6).
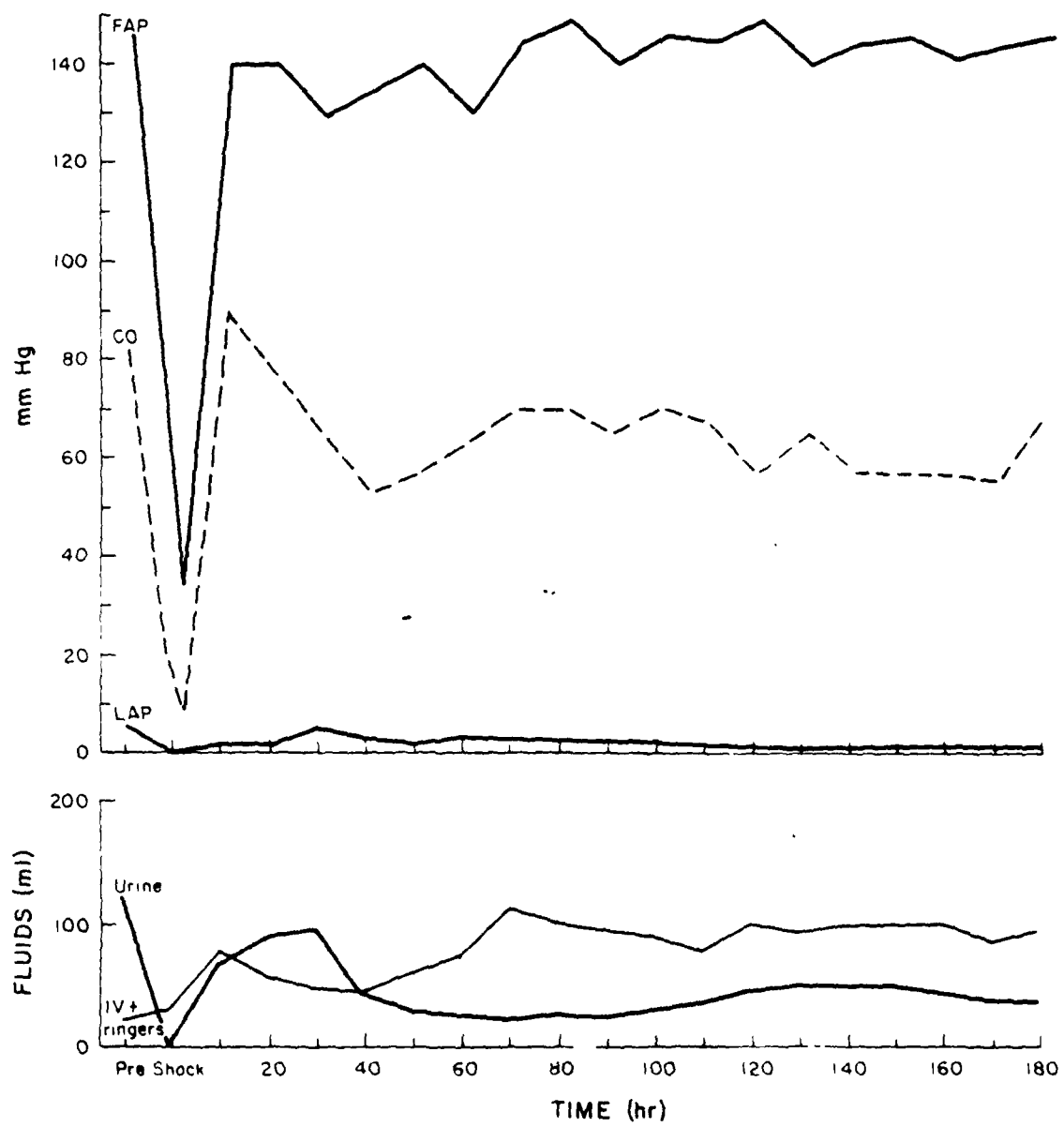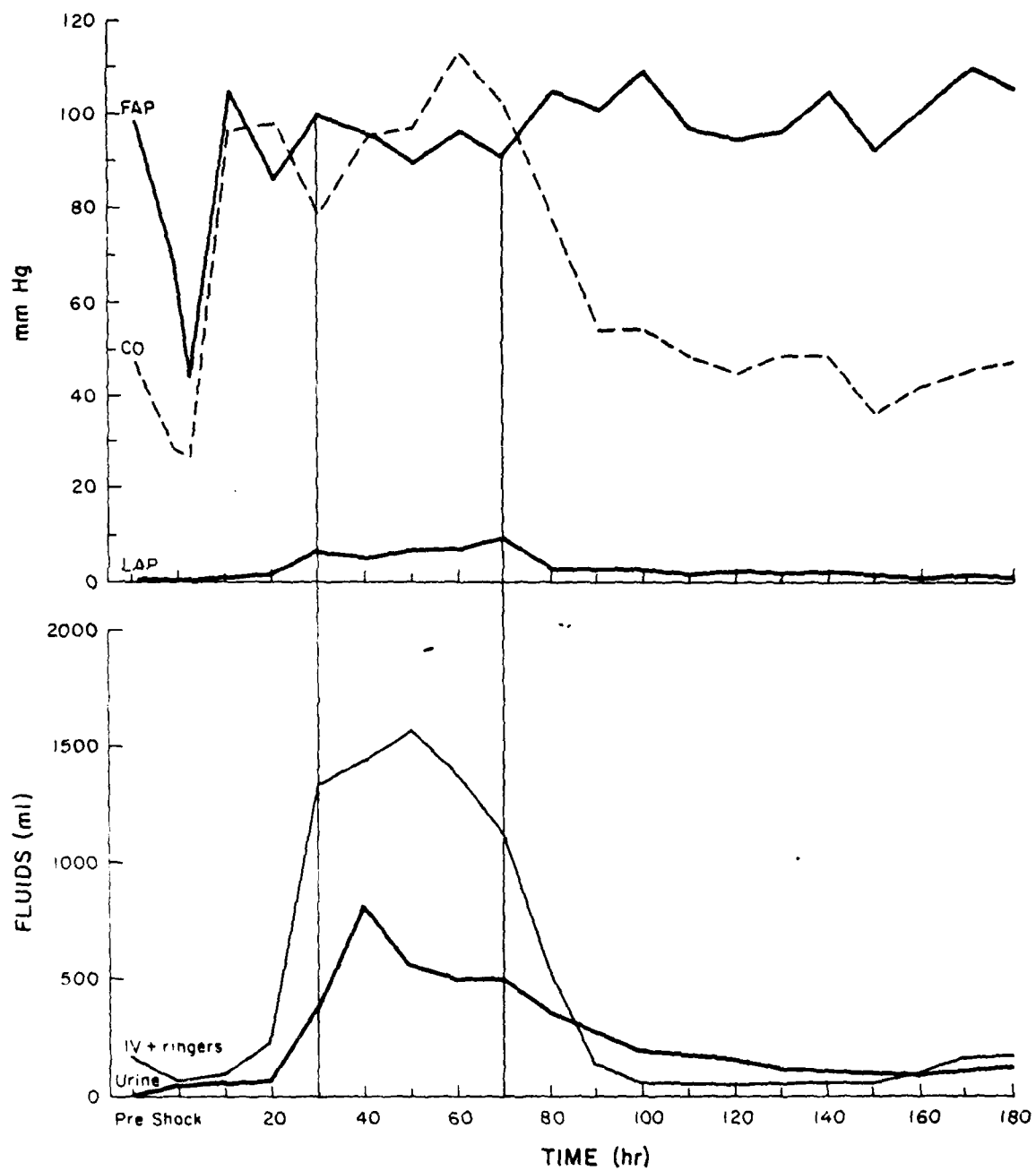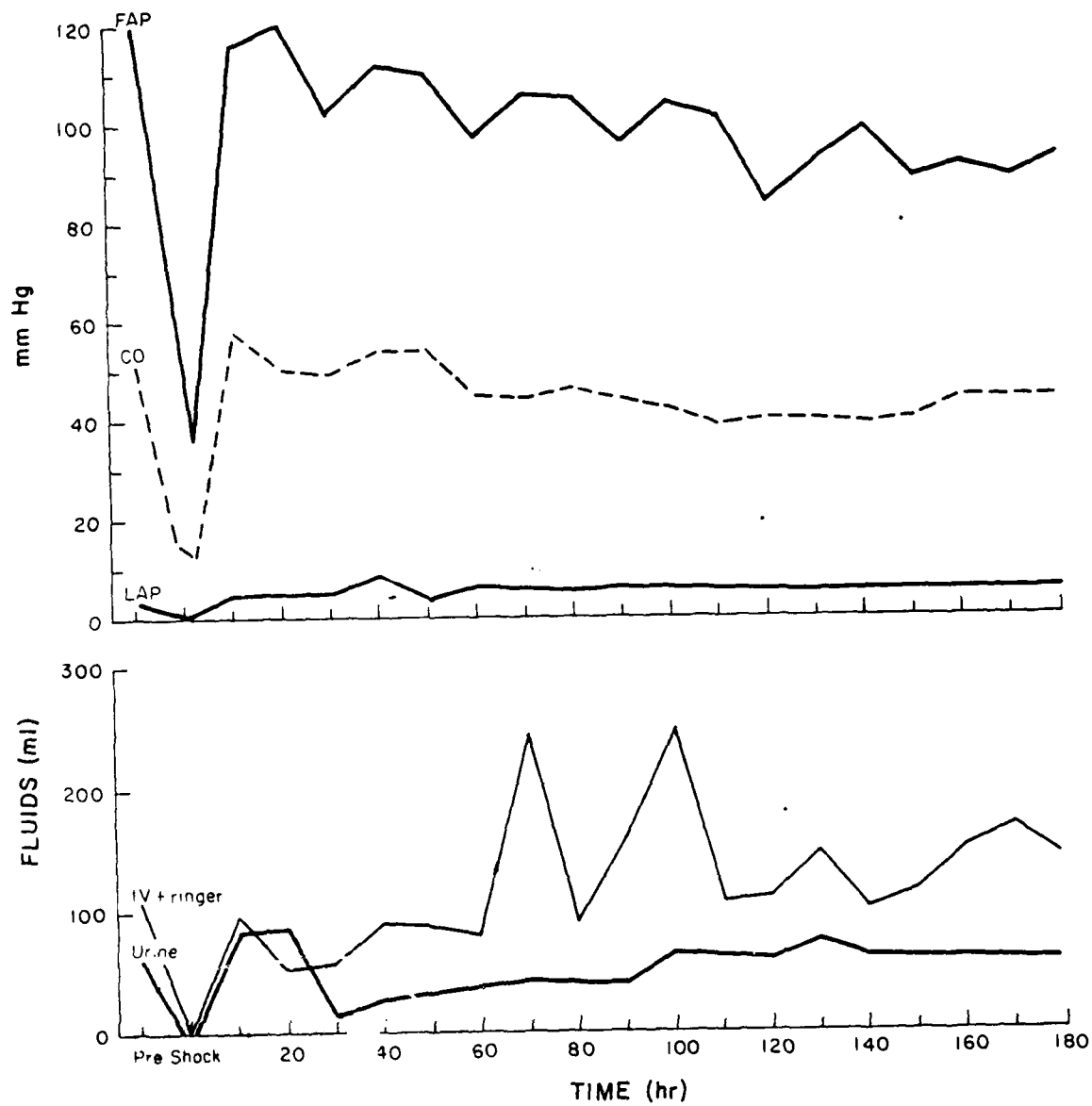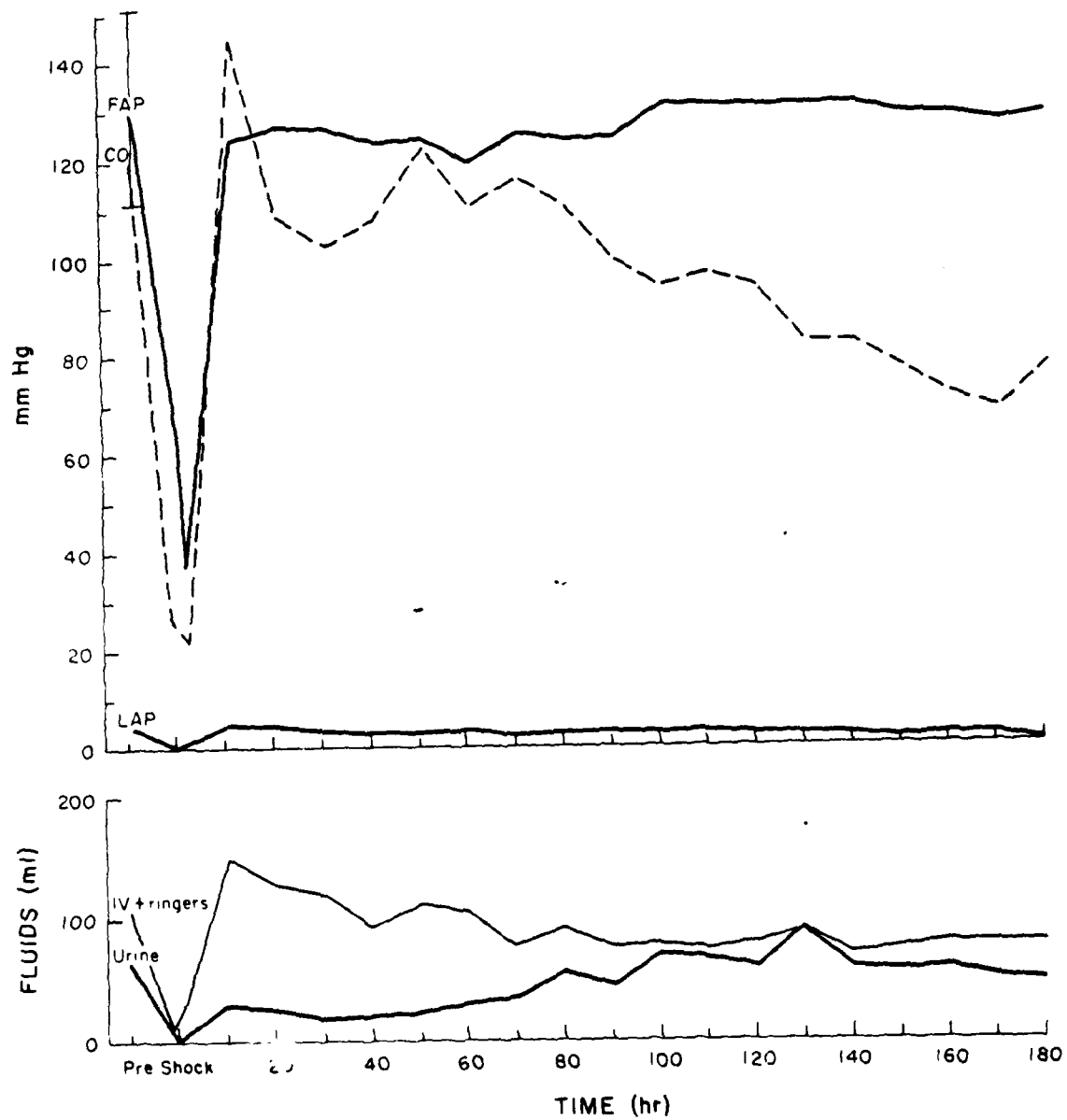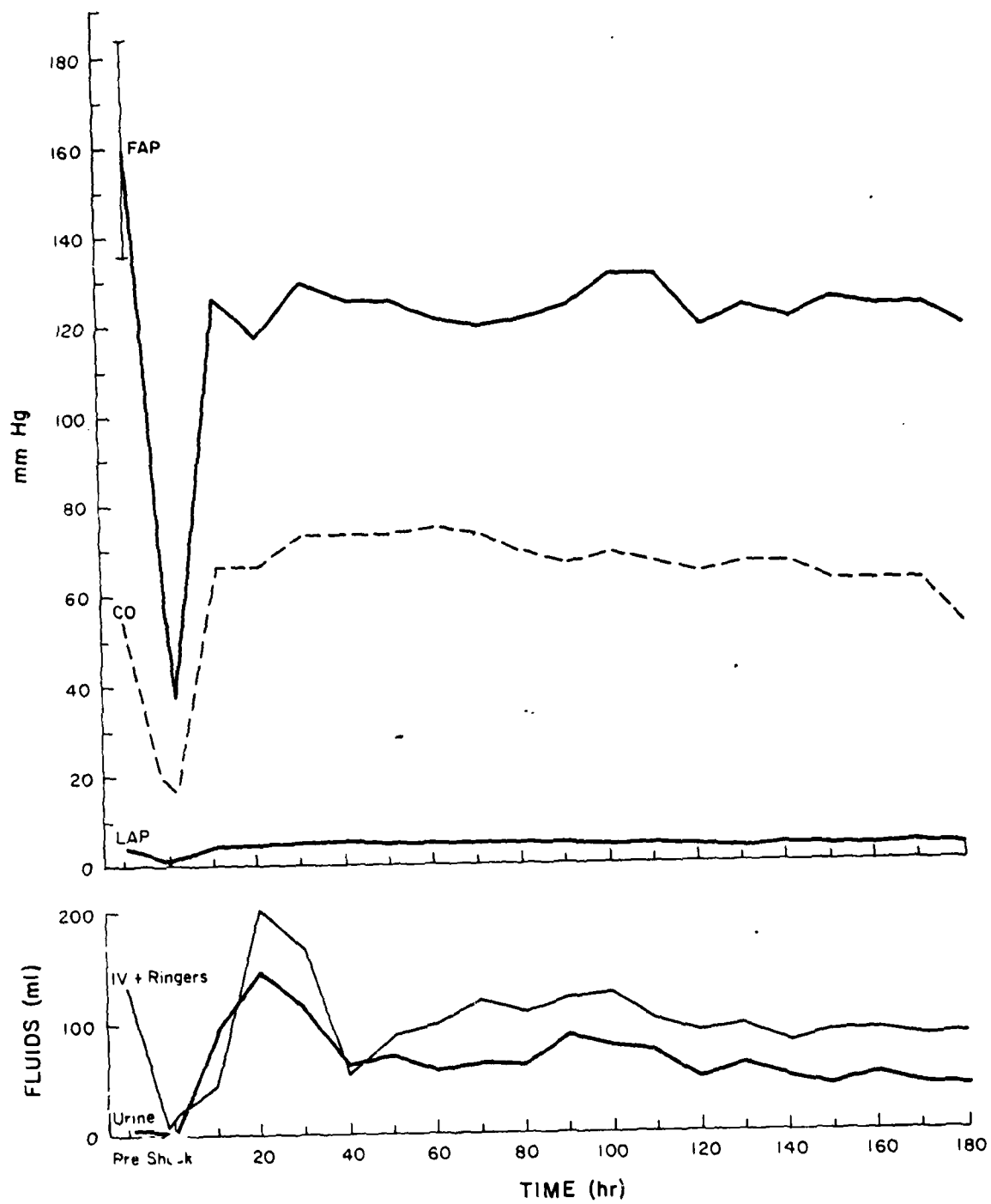
Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

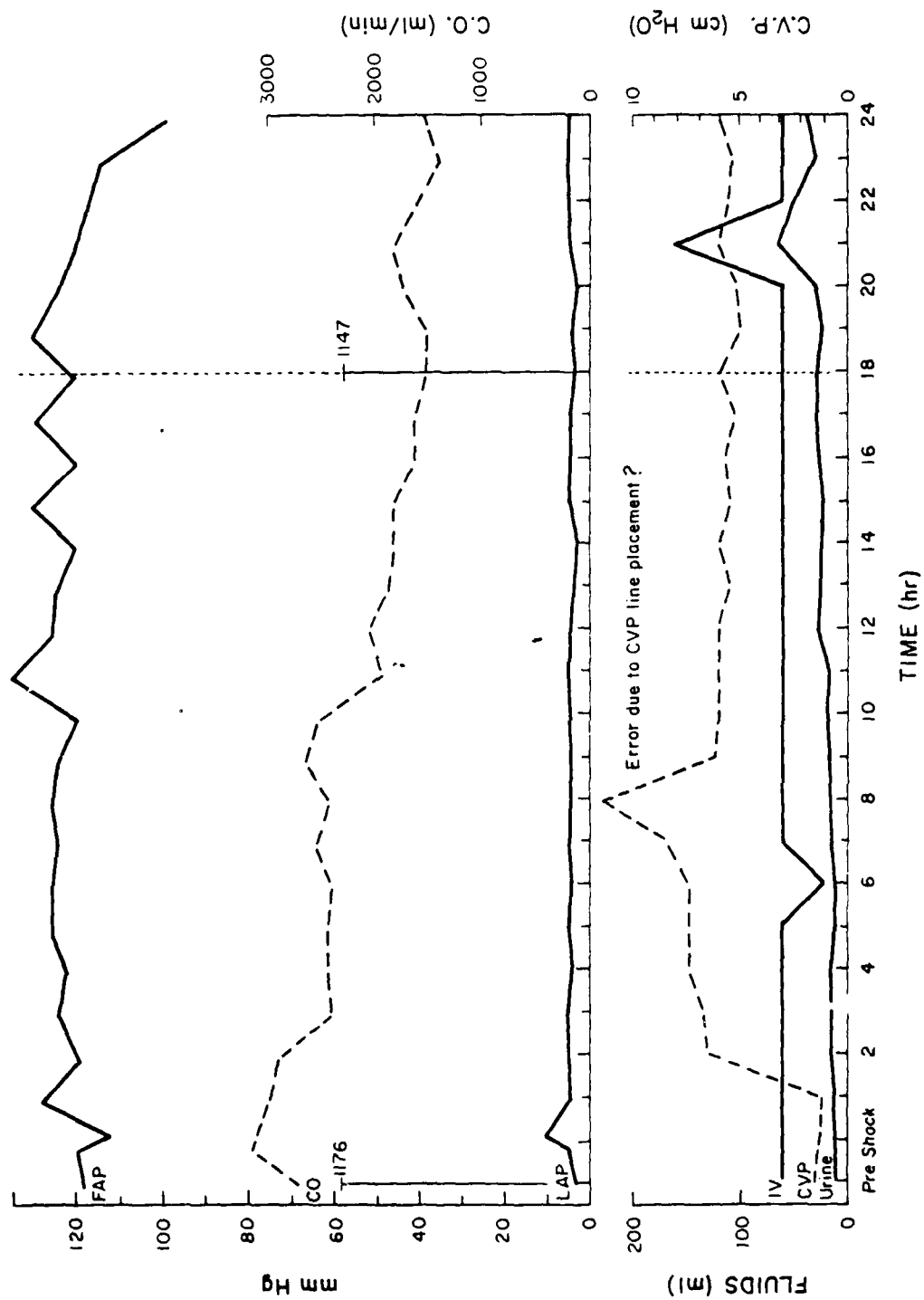Figure 6

Mechanisms of Shock Blood Induced Tissue Anoxia

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Disturbed cellular metabolism is a characteristic consequence of shock [1–4]. It is usually ascribed to the local effects of hypoperfusion and tissue anoxia. Our study was undertaken to determine whether other factors carried in the blood stream may accentuate and contribute to impaired cellular metabolism, dysfunction and death.

Material and Methods

Fourteen domestic pigs (30 to 45 kg) were starved overnight. They were sedated with ketamine hydrochloride (Ketalar®), 6 mg/kg, and anesthesia was induced by Pentothal® sodium intravenously. Tracheotomy was performed and the animals were connected to a Harvard large animal respirator. Anesthesia was maintained with 50 percent nitrous oxide in oxygen and intermittent diazepam intravenously (Roche Laboratories). The carotid artery was catheterized and used for monitoring arterial pressure, and the jugular vein was used for infusion of Ringer's solution. The right femoral artery and vein were catheterized as sources for withdrawal and reinfusion of blood during shock. Then 500 cc of venous blood was withdrawn slowly into heparinized syringes (16 units of heparin per cubic centimeter of blood). Volume was replaced with Ringer's solution at a rate sufficient to prevent a decrease in mean arterial pressure. The venous blood was used to prime a perfusion apparatus to be described later.

The left femoral vessels were isolated, catheterized and immediately connected to the perfusion apparatus. Isolation was effected by rapid amputation, and perfusion of the left hind limb was begun immediately. During perfusion the animal was bled into hemorrhagic shock for 1 hour. Mean arterial pressure was decreased to 60 mm Hg for 20 minutes and then 40 mm Hg for 40 minutes. The animal was then exsanguinated and the shed blood constituted a

shock blood perfusate used to prime a parallel perfusion system.

Perfusion system: The parallel perfusion system used (Figure 1) consisted of a venous reservoir closed to the atmosphere, a low flow perfusion pump that provided nonpulsatile flow (model 5M6232, Travenol Laboratories), a membrane oxygenator (model 0400-2A, Sci Med), a heat exchanger (model 5M0337, Travenol Laboratories), and Silastic® tubing. The heat exchanger was set at 37°C and the limb was wrapped in a warming blanket (model K-20, GRI Medical Products), also set at 37°C. Statham AA pressure transducers (model P23) were connected by side ports to the limb arterial and venous catheters and perfusion pressure was recorded on a Gould four-channel brush recorder (model 440). Gas flow through the membrane was maintained with oxygen and Carbogen® tanks.

Blood gases, pH, hemoglobin, glucose, lactate and electrolytes were monitored from arterial and venous samples from the pressure ports and were corrected when needed to maintain values within normal physiologic ranges.

Brace and Guyton [5], studying transcapillary fluid dynamics in isolated dog limb perfusion, noted that a perfusion pressure of 50 to 80 mm Hg was optimal in preventing fluid shifts in the isolated perfused hind limb. A series of preliminary experiments in which the limb weights were monitored confirmed that 50 mm Hg of perfusion pressure provided optimal steady state perfusion in pigs. With a constant perfusion pressure of 50 mm Hg, limb weight was maintained at a constant level and oxygen consumption was maximal. Therefore, steady state perfusion of the limb was established at whatever flow was necessary to maintain a constant perfusion pressure of 50 mm Hg.

As soon as the limb was amputated, perfusion was begun and blood gas, pH and glucose levels were normalized by adjustment of gas flow and injection of sodium bicarbonate and 5 percent dextrose into the venous reservoir as needed. Limb resistance normally decreased slowly over the first 20 minutes of perfusion until a steady state was established. When limb resistance reached a steady state, that flow value was considered the normal baseline. The shock blood
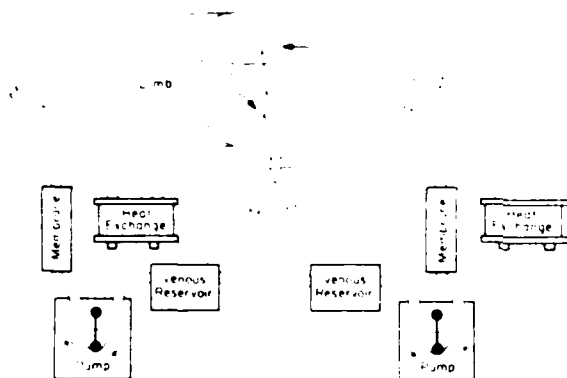
Figure 1. Schematic diagram of a parallel perfusion system.

from the exsanguinated animal was used to prime a parallel perfusion system (Figure 1) and was treated in the same manner. Switching perfusion from normal to shock blood and vice versa was effected by simply clamping or unclamping the appropriate Y in the tubing on both arterial and venous sides (Figure 1). The intrinsic volume in the limb and perfusion lines distal to the switching site constituted 10 percent of the total priming volume. This 10 percent mixing of normal and shock blood on switching to the alternate parallel system may have slightly diluted the impact of both the shock blood perfusion and the restoration of normal perfusion on the variables studied in the isolated limb.

In six control animals the limb was perfused with normal blood until steady-state normal flows were obtained. After 15 minutes of perfusion at normal flow, the flow was reduced to one-half normal (ischemic flow) and perfused for another 15 minutes, after which flow was returned to normal. Arterial and venous blood samples were taken and pressure and flow noted for each flow state for calculation of oxygen consumption, limb resistance, platelet count and platelet percentage aggregation to adenosine diphosphate. The protocol was repeated with shock blood and then perfusion was returned once again to normal flow with normal blood.

In eight additional pigs the same procedure was followed except that an alpha-adrenergic blocking agent, phentolamine (0.5 mg), was added to the venous reservoir of the perfusion system.

Treatment of data: Oxygen consumption was calculated from blood flow and simultaneous measurements of arterial and venous oxygen and carbon dioxide pressures and hemoglobin. Blood flow was determined from the precalibrated pump setting. Peripheral vascular resistance was calculated from the arterial and venous pressure differences through the limb and the precalibrated flow. Blood gases and pH were determined using a blood gas machine (model 127 and 113, Instrumentation Laboratories), and hemoglobin was obtained from a co-oximeter (model 182, Instrumentation Laboratories).

Platelet samples were prepared with unopettes (Becton, Dickinson) and counted in a Neubauer hemacytometer. Platelet aggregation to adenosine diphosphate was performed in a platelet aggregometer (model 300, Chronolog) and recorded on a strip recorder (model 702, Chronolog). Venous blood samples, 0.6 ml, were centrifuged at 175 g for 15 minutes to recover the platelet-rich plasma, and 0.02 ml of stock adenosine diphosphate (100 μg/ml) was injected into 0.4 ml samples of platelet-rich plasma. Percent aggregation was defined as the peak percent change in light transmission after the addition of adenosine diphosphate.

Controls: The results in six control animals are shown in Table I. Perfusion with shock blood produced a large and immediate increase in peripheral vascular resistance. In five of six limbs, resistance during perfusion with shock blood increased so much that normal flow could not be established and only "ischemic" flow could be maintained. Peripheral vascular resistance increased from 0.8 units of peripheral resistance (PRU) with normal blood at normal flow (NN2) to 2.0 PRU with shock blood at ischemic flow (Si) (p <0.0005). In contrast, peripheral vascular resistance for normal blood at ischemic flow (Ni) was not significantly different from that of normal blood at normal flow (NN1) (0.8 PRU for Ni compared with 0.9 PRU for NN1). Oxygen con-

TABLE I Blood Flow, Peripheral Vascular Resistance, Oxygen Consumption, Platelet Count and Platelet Aggregation in Six Control Limbs

Perfusion Time (min)	Condition	Flow (Q) (ml/min)	Peripheral Vascular Resistance (PRU)	Oxygen Consumption (V) (ml/min)	Platelet Count (venous) (× 1,000)	Platelet Aggregation to ADP
30	NN1	62 ± 19	0.9 ± 0.3	1.5 ± 0.?	334 ± 54	14 ± ?
45	Ni	30 ± 10	0.8 ± 0.4	1.2 ± 0.7	295 ± 55	76 ± 6
60	NN2	63 ± 15	0.8 ± 0.3	1.4 ± 0.6	352 ± 70	72 ± 6
80	Si	27 ± ??	2.0 ± 0.6	0.7 ± 0.5	241 ± 5?	72 ± ?
95	NN3	64 ± 19	1.0 ± 0.3	1.1 ± 0.4	298 ± 4?	7? ± 4

Mean values ± standard deviation for six control limbs. ADP = adenosine diphosphate. Ni = normal blood, ischemic flow. NN = normal blood, normal flow. Si = shock blood, ischemic flow.
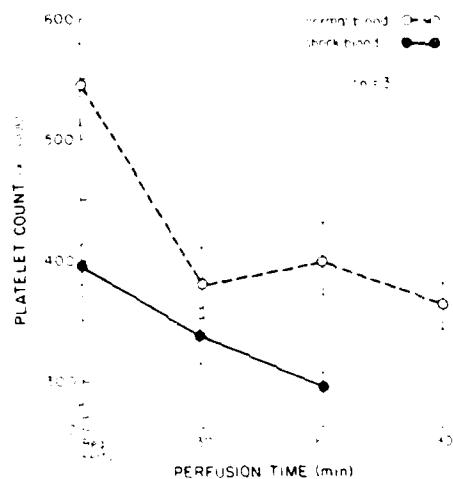
Figure 2. Platelet count over time during perfusion with normal blood compared with shock blood (mean ± standard error of the mean).

Figure 3. Peripheral vascular resistance in control limbs and phentolamine-treated limbs during perfusion with normal and shock blood (mean ± standard error of the mean). NN = normal blood/ normal flow; SI = shock blood/ ischemic flow; SN = shock blood/ normal flow.

sumption was significantly lower for perfusion with shock blood at ischemic flow (SI) (0.7 ml/min) compared with perfusion with normal blood at normal flow (NN2) (1.4 ml/min) (p <0.005) or normal blood at ischemic flow (NI) (1.2 ml/min) (p <0.05), or reperfusion with normal blood at normal flow (NN3) (1.1 ml/min) (p <0.05). Oxygen consumption and peripheral vascular resistance were not significantly different during reperfusion with normal blood (NN3) compared with NN1, NI or NN2.

In three control animals the platelet count was determined at the beginning of perfusion and at 30 minute intervals during perfusion for both shock and normal blood (Figure 2). In both shock and normal blood, the platelet count decreased significantly between the onset of perfusion and 1 hour of perfusion (p <0.05). In normal blood the platelet count appeared to level off after 30 minutes of perfusion, whereas in shock blood the platelet count continued to decrease with time. Platelet aggregation showed
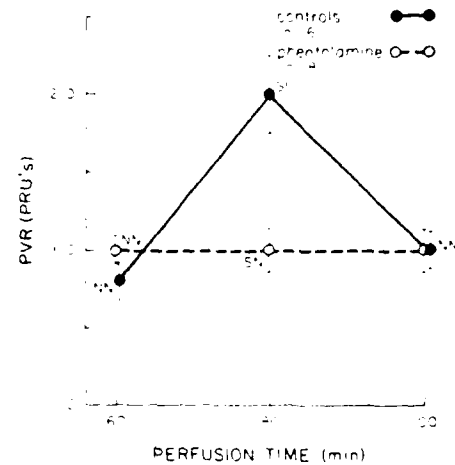
no change throughout any of the experimental states.

Fractionated plasma catecholamine levels were measured by radioenzymatic assay in one experiment in this series and showed an increase in epinephrine from 15 pg/ml in normal blood to 660 pg/ml in shock blood and a concomitant increase in norepinephrine from 54 to 1,580 pg/ml.

Administration of an alpha-adrenergic blocking agent: In eight additional pigs the same procedure was followed except that an alpha adrenergic blocking agent, phentolamine (0.5 mg), was added to the venous reservoir of the perfusion system. Phentolamine eliminated the increased peripheral vascular resistance seen with shock blood in every case (Table II and Figure 3). In two limbs the phentolamine was added to the system after shock blood perfusion had been established and the usual increase in peripheral vascular resistance had oc-

TABLE II Blood Flow, Peripheral Vascular Resistance, Oxygen Consumption and Platelet Count in Eight Phentolamine-Treated Limbs

Perfusion Time (min)	Condition	Flow (Q) (ml/min)	Peripheral Vascular Resistance (PRU)	Oxygen Consumption (VO₂) (ml/min)	Platelet Count (venous) (× 1,000)
60	NN1	54 ± 20	1.0 ± 0.3	1.3 ± 0.6	353 ± 92
80	SN	53 ± 18	1.0 ± 0.4	0.9 ± 0.5	242 ± 35
95	NN2	53 ± 20	1.0 ± 0.4	1.0 ± 0.5	273 ± 66

Mean values ± standard deviations for eight phentolamine-treated limbs. NN = normal blood/ normal flow, SN = shock blood/ normal flow
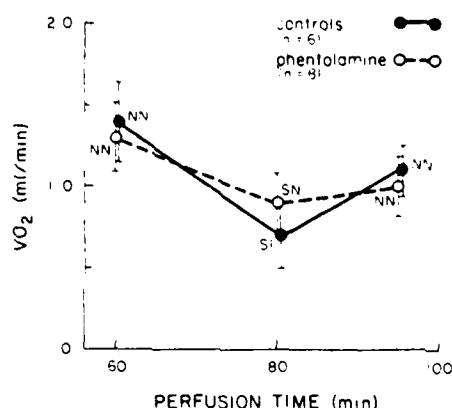
Figure 4. Oxygen consumption in control limbs and phentolamine-treated limbs during perfusion with normal and shock blood (mean ± standard error of the mean). NN = normal blood/ normal flow; SI = shock blood/ ischemic flow; SN = shock blood/ normal flow.

curred. The increase in peripheral vascular resistance was eliminated with phentolamine. In the remaining limbs the phentolamine was added to the normal blood before switching the perfusion to the shock blood and in these cases no increase in peripheral vascular resistance with shock blood was noted.

However, oxygen consumption remained lower in every case during shock blood perfusion despite normal flow rates (Table II and Figure 4). Mean oxygen consumption during shock blood perfusion was 0.9 ml/min compared with 1.3 ml/min for normal blood (NN1) (p <0.05). As in the control animals, oxygen consumption during reperfusion with normal blood (NN2) was not significantly different from initial levels (NN1). Platelet count was not significantly lower for shock blood than for normal blood.

Comments

Our study shows that perfusion of an isolated pig hind limb with shock blood results in a significantly increased peripheral vascular resistance that can be blocked by phentolamine. However, a significantly *reduced oxygen consumption seen during perfusion* with shock blood is not eliminated with phentolamine despite normal peripheral vascular resistance.

Reduced oxygen consumption was seen in every case during perfusion with shock blood. Oxygen consumption improved during reperfusion with normal blood and was not significantly different from initial values. Theoretical mechanisms for reduced oxygen consumption in shock blood include arte-

riovenous shunting and metabolic factors interfering with oxygen uptake or utilization.

Role of catecholamines: Increased plasma catecholamine levels in hemorrhagic shock were documented over 60 years ago by Bedford [6] and have been measured regularly since then in many forms of shock. Circulating catecholamines cause a decrease in clotting time, an increase in blood glucose, inotropic and chronotropic stimulation of the heart and cutaneous and visceral vasoconstriction.

As mentioned in the results, a 40-fold increase in the epinephrine level and an 80-fold increase in the norepinephrine level were measured in shock blood in one experiment in this series. The elevated catecholamine levels in shock blood were further evidenced by the significant increase in peripheral vascular resistance during perfusion with shock blood, which can be entirely blocked with phentolamine. Reduced oxygen consumption in the presence of elevated plasma catecholamine levels can be adequately explained in the control animals by reduced blood flow due to vasoconstriction. It obviously does not explain the observed reduced oxygen consumption in the presence of normal blood flow in phentolamine-treated animals.

Other factors: A variety of other factors have been postulated to explain reduced oxygen consumption in shock. These include platelet aggregation, changes in blood affinity for oxygen and a variety of humoral factors such as histamine, serotonin, bradykinin, kallidin II, angiotensin II, adenosine, prostaglandins, thromboxanes, smooth muscle acting factor [7], acid hydrolases [8], myocardial depressant factor [9], lysosomal enzyme [10], splanchnic ischemic factor [10], plasma factor [10] and cardiotoxic material [11]. Which of these factors may be responsible for the findings in our study is not known.

Deterioration in cell membrane function has been documented after shock [12]. Appelgren [13] investigated severely depressed tissue-blood exchange in the presence of apparently adequate whole organ flow. He studied the relative roles of perfusion and diffusion in the passive exchange of low molecular substances between the cell and blood in skeletal muscle in hemorrhagic shock in dogs. He concluded that tissue-blood exchange is disturbed in shock because of both a reduced perfusion and a reduced diffusion, but that reduced diffusion may be interpreted as simply due to a reduction in the number of perfused capillaries in shock or an uneven capillary perfusion. He suggests that the distribution of flow through capillaries is regulated separately from the total blood flow, although the mechanism for mal-

distribution during shock is not known. Arteriovenous anastomotic blood flow has been responsible for a similar phenomenon observed in the septic hind limb of dogs [14] but has not been investigated in hemorrhagic shock. Arteriovenous shunting then is another reasonable explanation for the observed reduction in oxygen consumption.

Our study shows that oxygen consumption in shock is significantly reduced even in the presence of apparently normal blood flow. The mechanisms involved in the observed reduction of oxygen consumption are purely speculative and subject to further study.

Summary

An isolated limb perfusion system in pigs was used to assess and relate hemodynamic variables to oxygen consumption in shock. Perfusion with shock blood results in a significantly increased peripheral vascular resistance, which can be blocked by phentolamine. However, a significantly reduced oxygen consumption seen during perfusion with shock blood is not eliminated with phentolamine despite normal peripheral vascular resistance. The cause of the reduced oxygen consumption is not known, but the results suggest that other factors apart from a decreased blood flow due to catecholamine release are important in contributing to end organ anoxia and failure in shock.

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Discussion

John Benfield (Duarte, CA): Dr. McNamara and his associates have made considerable progress in developing a useful model with which to study shock. They have shown about a 2.5-fold increase in vascular resistance and roughly a one third to one half reduction in oxygen uptake in a pressure-regulated perfusion system in pigs when the perfusate is blood from an animal in shock. They have also shown that the hemodynamic consequences can largely be neutralized with an alpha-adrenergic blocking agent, but that phentolamine did not reverse the metabolic consequences that they measured.

Although one of their key references is dated more than half a century ago, clinical surgeons have begun to think and act about shock in metabolic terms only relatively recently. I am referring to the still controversial issues of the use and abuse of corticosteroids or vasodilators for treatment. These therapies reflect our recognition that the important consequences of shock are its effects on cell metabolism and perhaps the cell membrane, and that variables such as vascular resistance and oxygen uptake are best viewed as indicators of events at the cellular level. For example, fundamental work such as this makes us question just how much oxygen is actually being delivered to the cells and what humoral factors may be acting on the cell membrane. How much unevenness of blood flow or shunting is associated with perfusion of an isolated limb? I raise these questions not expecting any answers today, but rather as a projection of goals for the future.

The preparation Dr. McNamara and his group have studied is a demanding one, making it difficult to always obtain the theoretically desired controls. For example, the effects of adrenergic blockade were studied in one group and not in another and perfusions with normal blood seemed always to precede trials of perfusion with shock blood. I wonder if we know the effects of perfusion with shock blood had been done first. More simply stated, can you tell us to what degree your preparation lends or does not lend itself to internal controls or how long a period of reliable observation is possible after each preparation is made?

Donald D. Trunkey (San Francisco, CA): One interpretation of your data is that this is a physical rather than a physiologic phenomenon, particularly in regard to diffusion. Any increase in interstitial swelling would cause an increase in the diffusion distance. Did you make any specific measurements in that regard? Did you weigh the hind limb preparation before and after its control and shock

perfusion or did you take any other measurements to document an increase in the interstitial edema, or both?

Another interpretation of your data is that you simply have not provided substrate. In other words, oxygen consumption is also dependent on the state of respiratory activity and whether or not there is a change in the ATP-ADP-O_2 ratios. Did you measure that also?

Nicholas A. Halasz (San Diego, CA): One of my questions is related to what Dr. Trunkey referred to, interstitial pressure. In addition to measuring weight, it is now fairly easy with wicks inserted into the subfacial plane to actually measure interstitial pressure. I would be most interested in the results of such studies now or in the future.

The second question has to do with another potential explanation of this peculiar change in oxygen uptake, that is, is it simply a matter of a change in hemoglobin? It would be interesting to know what level the venous oxygen pressure decreased and, specifically, whether P_aO_2 was determined.

J. Judson McNamara (closing): A few general comments that to some extent were alluded to by the first discusser, Dr. Benfield. Since the importance of fluid replacement in hemorrhagic shock was recognized in the 1930s and 1940s, attention has been focused on the multiple organ failure syndrome. These problems are seen after effective hemodynamic resuscitation from shock including pulmonary insufficiency, renal failure, stress ulcer, surgical metabolism, liver failure and depression of immune mechanisms. All of these complications are directly related to the severity of the initial insult. It therefore becomes apparent that we should not disregard the need to continue to investigate the mechanism of and improved therapy of the initial insult, the episode of shock. In other words, can we more rapidly restore nutrient tissue blood flow to normal?

We have shown recently in another model that visceral blood flow, that is, spleen, portal and intestinal flow, and bronchial flow remain suppressed for as long as 18 hours after resuscitation to baseline values of animals in hem

orrhagic shock. The current study shows that some metabolic defect still presists in an adequately perfused limb when it is perfused with shock blood. This element may also interfere with restoration of normal nutrient blood flow of the tissue. My point is that there is still much to uncover about the acute resuscitative phase of shock and the kinds of things that we can do to actually restore nutrient blood flow to normal.

Now to answer some specific questions, Dr. Benfield raised an important point regarding the model. We can get about 3 useful hours of a steady state perfusion system. In this particular experiment, after we have established a steady state and bled the animal into shock for 1 hour, we do not have time for much manipulation with the system before deterioration of steady state begins.

Dr. Trunkey asked some very cogent questions about the status of interstitial fluid in the limb. We have measured and monitored limb weights. We know that limb weight is stable but have not looked at interstitial edema specifically. The changes in vascular resistance and oxygen consumption are immediate, so I doubt that tissue edema and interference with oxygen transfer play a role. Similarly, we have not specifically investigated changes in oxygen P_aO_2 as another possible mechanism of reduced oxygen consumption, although Dr. Alden Harken noted some decrease in oxygen consumption with a decrease in P_aO_2, but not in the order of magnitude we saw. Finally, we have no measurement of actual substrate supply.

We have, however, identified the effect of shock blood on the delivery of oxygen to the tissue in this particular model. We are most interested in the presence of arteriovenous shunting. We have studied three or four animals and have been able to demonstrate excessively rapid appearance of dye in the venous perfusion, suggesting that there is arteriovenous shunting. We decided to address arteriovenous shunting as the first possible mechanism of decreased oxygen consumption because other data in the literature suggest that this is a mechanism and it has been identified as a mechanism for reduced oxygen consumption in septic shock. We hope to have the opportunity to present some of these additional data in the future.